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Seed storage proteins in *Solanaceae* and *Cucurbitaceae* species

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Abstract

Electrophoretic analyses of non-reduced and reduced seed storage proteins from *Solanaceae* and *Cucurbitaceae* species and cultivars were performed. High molecular disulfide bonded complexes between intermediary subunits of 11S globulins previously detected in *Capsicum annuum* cultivars, were found in *Solanum melongena* cultivars as well. The data obtained might be used for further elucidation of peculiarities of the 11S globulins in dicotyledonous plants.

Additional key words: *Capsicum annuum*, eggplant, electrophoretic spectra, globulins, intermediary 11S subunits, oligomers, pepper, *Solanum melongena.*

Seed proteins are widely used in plant genetic studies (Cooke 1995, El Naggar 2001, Jha and Ohri 2002, Syros *et al*. 2003). The seed storage proteins in dicotyledonous plants are oligomeric globulins, presented mainly by two size classes: 7 to 8S, vicilin-like and 11 to 14S, legumin like proteins. The 7S globulins, Mr 150 - 180 kDa, consist of three subunits joined together via weak interactions: hydrogen and hydrophobic bonds. The 11S globulins, Mr 350 - 460 kDa, are hexamers in which each subunit, the so called intermediary subunit, is composed of a disulphide bonded acidic (40 kDa) and basic (20 kDa) polypeptide (Derbyshire *et al.* 1976, Higgins 1984, Shewry *et al.* 1995). In some dicotyledonous plants, *e.g.* pumpkin (Hara-Nishimura *et al.* 1993) and sunflower (Anisimova *et al.* 1995), a third type seed storage proteins 2S albumins, are available in abundance. Under non-reducing condition, when only denaturing and solubilizing agents (urea and sodium dodecyl sulphate) are used, 7S globulins dissociate into consisting subunits and 11S globulins into intermediary 11S subunits. After reduction, disulphide bond between acidic and basic polypeptides breaks up and acidic and basic polypeptides appear in the electrophoretic spectrum. In previous investigations we found that non-reduced 11S globulins in pepper cultivars contain not only intermediary 11S subunits, but oligomeric complexes between them, as well (Vladova *et al.* 2000a,b, 2002). We did not find such oligomers in the spectra of the cultivars from two other *Solanaceae* genera - *Nicotiana* and *Lycopersicon* (Vladova *et al.* 2000a) and in the electrophoretic spectra of two *Cucurbita* species: *C. maxima* (pumpkin) and *C. pepo* (vegetable marrow) (Vladova and Petkolicheva 2001). Similar oligomers have been observed only in a few other dicotyledonous plants (Hagger and Dank 1996).

The aim of the present investigation is to screen some economically important species from *Solanaceae* and *Cucurbitaceae* families for the presence of oligomers between the 11S intermediary subunits in the electrophoretic spectra of their non-reduced seed storage proteins.

Seeds from the following species and cultivars are used in this study: *Solanaceae*: *Capsicum annuum* L. (pepper) cvs. Hebar and Albena, *Solanum melongena* L. (eggplant) cvs. 12 and Lych, *Lycopersicum esculentum* Mill. (tomato) cv. Miglena; and *Cucurbitaceae*: *Cucurbita maxima* (pumpkin) cv. 696900 and *C. pepo* (vegetable marrow) cv. Izobilna, *Cucumis sativa* L. (cucumber) cv. Gergana and *C. melo* (melon) cv. Medena Rosa, and *Citrulis edulis* (water melon) cv. Mramorna. The seeds were ground into a fine powder and deffated with 30 $cm^3 g^{-1}$ cold acetone. 0.010 g of air-dried flours were mixed with 0.5 cm^3 0.05 M Tris-HCL buffer, pH 8.0, containing 2 % SDS and 5 M urea. The slurries were vortexed periodically and after 2 h at room temperature were centrifuged in an *Eppendorf 5402* (Hamburg, Germany) centrifuge at 15 800 *g* at 4 °C for 15 min. The

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Abbreviations: SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis; 2-ME - 2-mercaptoethanol, Mr - relative molecular mass.

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clear supernatants (6 mm^3 for *Solanaceae* species and 3 mm^3 for *Cucurbitaceae* species per well) were used for electrophoretic analysis. Home made, dual gel unit for vertical slab gel electrophoresis (gel size 70/60/0.5 mm and 12 wells) was used. Reducing conditions were achieved by addition of 2-mercaptoetanol (2-ME) to the protein extracts (6 mm^3 2-ME were added to 100 mm^3 extract). Electrophoretic separation was carried out on 12.5 % SDS-PAGE, pH 8.8, according to Laemmli (1970) with modifications (Vladova *et al.* 1989). The protein components were visualized with Coomassie Brilliant Blue R 250. The following proteins were used as molecular mass markers: myosin (205.0 kDa), galactosidase (116.0 kDa), phosphorylase *b* (97.0 kDa), fructose-6-phosphate kinase (84.0 kDa), albumin (66.0 kDa), glutamic dehydrogenase (55.0 kDa), ovalbumin (45.0 kDa), glyceraldehyde-3-phosphate dehydrogenase (36.0 kDa), carbonic anhydrase (29.0 kDa), tripsinogen (24.0 kDa) and trypsin inhibitor (20.0 kDa).

The electrophoretic spectra of non-reduced seed storage proteins of all investigated cultivars contain intermediary 11S subunits and components of 7S globulins (Fig. 1a - j). Clearly marked high molecular components are observed only in the spectra of *C. annuum* and *S. melongena* cultivars (Fig. 1a,b,c,d). Components in the electrophoretic spectra of *C. annuum* cultivars (Fig. 1a,b) are denoted according to the diagram presented in our previous publication (Vladova *et al.* 2000a). Component N 1 (Mr 116 kDa) is common for the two pepper cultivars. Component N 2 (Mr 94 kDa) is observed in cv. Hebar (type *a*), component N 2 (84 kDa) in cv. Albena (type *b*). Both investigated eggplant cultivars exhibit the same phenotypes of electrophoretic spectra of their non-reduced seed storage proteins. They contain three high molecular components. Two of them (N 1s and 2s) are minor with Mr above 116 kDa. The third one is well marked and its Mr (116 kDa) coincides with that of the N 1 component in the spectra of pepper

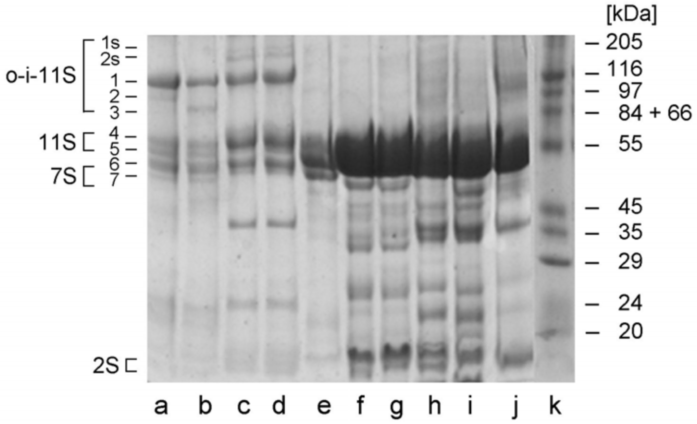

Fig. 1. Electrophoretic spectra of non-reduced seed storage proteins in: *C. annuum* cvs. Hebar (a) and Albena (b); *C. melongena* cvs. 12 (c) and Lych (d); *L. esculentum* cv. Miglena (e); *C. maxima* cv. 6969 (f); *C. pepo* cv. Isobilna (g); *C. sativa* cv. Gergana (h); *C. melo* cv. Medena Rosa (i); *C. edulis* cv. Mramorna (j); Mmm (k). o-i-11S - oligomeric complexes of 11S intermediary subunits; 11S - intermediary subunits of 11S globulins; 7S - components of 7S globulins; 2S - components of 2S storage albumins.

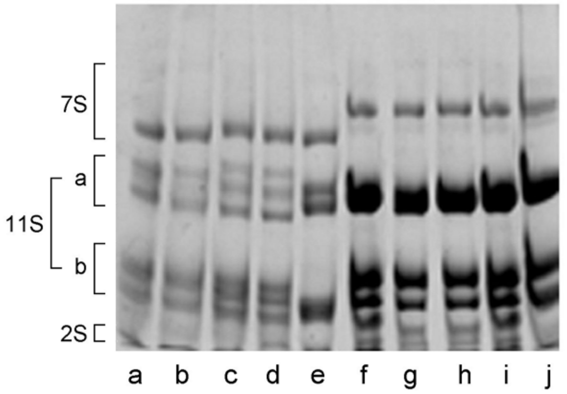

Fig. 2. Electrophoretic spectra of reduced seed storage proteins. The denotations are the same as in Fig. 1. a and b - acidic and basic polypeptides of 11S globulins, respectively; 7S - components of 7S globulins; 2S - components of 2S storage albumins.

cultivars (Fig. 1a,b,c,d). Further investigations will elucidate whether this fact is connected with some more common particularities of 11S globulins in *Capsicum* and *Solanum* species. Some minor, diffuse spread high molecular components are observed in the electrophoretic spectra of other investigated crops (Fig. 1e - j). Our former results showed that after reduction oligomeric 11S globulins in pepper cultivars disappear due to their dissociation into acidic and basic polypeptides (Vladova *et al.* 2000a,b). Therefore, the reduced proteins of the crops studied were analyzed electrophoretically, as well. No high molecular components are observed in the resulting electrophoretic spectra (Fig. 2a - j). So, it might be assumed that all high molecular components presented in the non-reduced spectra are disulphide-bonded monomers, most probably acidic and basic polypeptides of the 11S globulins. It is interesting to mention that Mrs of 7S globulins in the spectra of *Cucurbitaceae* crops are considerably higher than those of *Solanaceae* crops. Another common attribute of investigated members of *Cucurbitaceae* family is the presence of 2S storage albumins in their electrophoretic spectra of non-reduced (Fig. 1f - g) and reduced (Fig. 2f - j) seed proteins.

To conclude, as a result of the investigation carried out one more crop (besides *Capsicum annuum* L.) - *Solanum melongena* L. in which the intermediary 11S subunits join into intermolecular complexes has been found. The data obtained might be used for further elucidation of the peculiarities of the 11S globulins in dicotyledonous plants.

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